The use of different objects during a Novel Object Test in stabled horses

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ABSTRACT

Novel object tests are often used to evaluate a horse's temperament by recording fear responses towards a unfamiliar object but they might also be used as a part of welfare assessment. Various objects are used during these tests. This study aims to verify the use of different objects during a novel object test performed in the horse's stall. To this end, fifty-four horses and four objects (red-white striped cone: RWCONE; a red-yellow plastic ball: BALL; a black open umbrella: UMBR; black-yellow striped cone: BYCONE) were selected. In order to verify associations between behavior during the novel object test and undisturbed behavior at stable, baseline behavior profiling (4 x 10 min, spread over four days) was carried out. Thereafter, novel object tests were performed. Each object was presented for 10 minutes to each horse in their stall, spread over four consecutive days. Each horse was exposed to the four objects in a semi-random order. The results reveal a higher frequency of object related behavior in the presence of the umbrella ($P = 0.0005$), which might be caused by the color and the size of the object. No differences in object related behaviors were found between the two cones which were colored differently. The age of the horse must be taken into account, as younger horses showed more pronounced reactions to the objects. Sniffing behavior towards the object positively correlates with specific features of housing (large stall, visual and physical contact with other horses). A higher frequency of fear reactions to the objects is associated with more vocalizations during undisturbed behavior observations, which might be indicative of stress. The results show that associations between behavior during the novel object test and behavior at stable were present. However, the presence of roughage influences the horse's reaction and should be taken into account. In this case, the focus should be on the presence of fear reactions, such as rearing, not approaching the object or defecating, in order to correctly determine the animal's welfare state.

Keywords: horse, novel object, behavior

INTRODUCTION

Welfare of stabled horses has become increasingly important and the way of housing and managing horses is often questioned (Visser et al., 2008). There are different ways to assess welfare as for example the presence of abnormal behaviors and stereotypies has been linked to poor welfare conditions (Mason and Latham, 2004). In horses, inadequate housing and management, such as confinement and restricted fiber meals, might lead to the development of these abnormal behaviors (McGreevy et al., 1995). However, their presence does not always indicate a lower welfare level at that moment (Mason, 1991). In addition, the presence of stress also relates to horses' welfare. Physiological responses to stress are seen, as heart rate and salivary cortisol concentrations both increase (Kiley-Worthington, 1990; Bagshaw et al., 1994). However, the latter techniques to measure stress require direct contact with the horse and consequently affect the measurements.

Novel object tests are often used to test the horse's temperamental characteristics, for example for breeding purposes. They might also be used to select the right horse for a specific rider (Visser et al., 2002). However, as results from previous research already suggested, the response of a horse during a novel object test relates to its behavior at stable (Visser et al., 2008). This test can also be used to assess welfare. Animal welfare assessment is based on different measurements, including behavioral and physiological measurements, with a focus on health and behavior. Various measurements are necessary in order to make up a complete picture of the animal's welfare (Wageningen UR Livestock Research, 2011).

Researchers from Wageningen UR Livestock Research developed a protocol to assess welfare in horses and they did include the novel object test in their protocol (Wageningen UR Livestock Research, 2011). Horses tend to avoid potentially fear-eliciting situations and they tend to respond nervously to novelty in a known environment. Responses in novel object tests

may reflect exploratory motivation, play behavior, fearfulness, emotionality or no interests in the object (Wood-Gush and Vestergaard, 1991; Christensen et al., 2005). The advantage of this way to measure welfare is that behavior is easy to measure and signs of fear are relatively easy to detect (Leiner and Fendt, 2011). However, during this test, horses are led out of their stable and exposed to a novel object in another environment. This implies environmental changes, such as social isolation and handling of the horse, which both possibly influence the results (Malmkvist et al., 2012). By performing the test in the horse's stall, these factors of influence are avoided and necessary test time is reduced, which are key factors in using this test as a part of welfare assessment. Furthermore, various objects are used during these tests and, to our knowledge, there is only little information available about differences between objects.

The aim of this study was therefore to verify if the novel object test can be performed in the stall of a horse and to verify whether horses react different towards different objects. During these tests, horses might be exposed to a combination of suddenness and novelty, but we focused only on the effects of novelty in a known environment (Christensen et al., 2005). Furthermore, we want to study associations between behavior during the novel object test and behavior at stable. The impact of housing and management factors is also measured.

MATERIALS AND METHODS

Animals and housing

Fifty-four horses (31 geldings and 23 mares) were selected from five riding schools (Table 1). Horses aged between 2 and 22 years, were used for riding school activities (dressage, jumping, …) and housed individually in stalls on straw or straw flax bedding. As feeding regime and housing slightly differed between riding schools, environmental parameters

(bedding material, feed, stall size and type of social contact) were recorded during each observation period (Table 2).

Objects

Four single objects of different color and shape were chosen. The objects were a red-white striped cone (RWCONE; 30 x 47 cm), a red-yellow plastic ball (BALL; 50 cm), a black open umbrella (UMBR; 90 cm) and a black-yellow striped cone (BYCONE; 30 x 47 cm). The novel objects were not used by the current horse owners but individual experiences in the past with similar objects cannot be excluded.

Experimental procedure

We tested reactions to the objects presented in the horses' home environment (stall). Novel object tests are often performed in a test arena, including handling horses and social isolation. These influence factors are avoided when performing the novel object test in the stall of the horse. Very small sized stalls were not taken into account. For this experiment the horses must have the opportunity to ignore or go backwards when they are confronted with the novel object. The experiment was carried out from August till October 2013.

Baseline behavior profiling was carried out before novel object tests were started. Both were performed by the same observer (Table 3). Baseline behavior profiling was carried out on four consecutive days. Each horse was observed for 10 minutes per day in its stall by the same observer. The prevalence of each behavior, as well as the start and end of each bout, during these 10 minutes was noted. Direct observation was chosen, as it allowed us to collect the most detailed behavioral information. Baseline behavior profiling was carried out between 11:00h and 21:00h, equaling a total of 36 hours of observations. For each horse, undisturbed behavior observations and novel object tests were started at the same time of day.

During the novel object test, one stationary object was placed at the floor close to the front stall wall equidistant from the two side walls. The umbrella was opened out of sight of the horse before placing it in the stall. This object was presented with the handle facing the horse. The object remained in the horse's stall for the next 10 min, during which behavior was recorded continuously by an observer, standing 1 m outside the stall (Malmkvist et al., 2012). The four objects were tested on four consecutive days. Each horse was exposed to the four objects in a semi-random order. Each horse was exposed to the objects in a different order, but overall each object was presented in each order the same number of times.

Data analysis

Data were analyzed using available procedures in SAS (Statistical Analysis Systems Institute, Cary, USA).

Differences in behaviors towards different objects were analyzed using the MIXED procedure, with the type of object as a fixed factor. Horse's age (2 – 22 years), gender (gelding, mare), stall size (1 – 3) and social aspect of the stall (1 – 2) were included as covariates in the start models. These models were reduced by stepwise removal of insignificant ($P > 0.05$) terms, keeping the main fixed factor (object type) in the final model (Malmkvist et al., 2012). Relationships between undisturbed behavior at stable and behavior towards the objects during novel object tests were analyzed using Pearson correlations (Visser et al., 2008). A significance level of 0.05 was used. Data are presented as percentages in the results (± S.E.M.).

RESULTS

Behavior during novel object tests

Total behavior related to the objects differed ($P < 0.0001$), with a higher frequency in the presence of UMBR compared to the other objects. The frequency of object related behavior during all object tests was related to by the age of the horse ($P = 0.0002$), the presence of feed ($P = 0.0193$) and stall size ($P = 0.0120$). Younger horses showed more behavior towards the objects compared to older horses ($r = -0.28$, $P = 0.0367$). A less pronounced reaction towards the object was observed in horses housed in a stall with medium size compared to horses housed in small or large stalls.

Looking at behaviors towards the objects separately, it is seen that they all occurred more in the presence of UMBR ($P = 0.0005$), excluding nibbling the object (Figure 1). Nibbling the object was observed more frequently in horses housed in small stalls. Sniffing the object occurred to a higher frequency in horses housed in larger stalls. Horses housed in stalls with visual and physical contact with other horses (stall type 2) showed more sniffing behavior towards the objects than horses housed in stall type 1 (only visual contact) (Table 4).

Associations between reaction to objects and undisturbed behavior at stable

Interacting indirectly with the object (focus, snort, rear) was associated with more vocalization during undisturbed behavior observations. This relationship was present with all objects (RWCONE: $r = 0.772$, $P < 0.0001$; BALL: $r = 0.763$, $P < 0.0001$; UMBR: $r = 0.439$, $P = 0.0009$; BYCONE: $r = 0.7488$, $P < 0.0001$).

The frequency of defecating in the presence of RWCONE was positively correlated ($P = 0.0021$) with the frequency of vocalizing during undisturbed behavior observations ($r = 0.4103$). The same correlation was seen in the presence of UMBR ($r = 0.505$, $P < 0.0001$) and BYCONE ($r = 0.401$, $P = 0.0032$).

For all objects, a positive relationship was seen between touching the object and bedding related behavior during undisturbed behavior observations (RWCONE: $r = 0.382$, $P = 0.0052$;

BALL: r = 0.283, P = 0.0381; UMBR: r = 0.451, P = 0.0006; BYCONE: r = 0.272, P = 0.0485). The opposite result is seen between touching the object and hay related behavior (RWCONE: r = -0.336, P = 0.0140; BALL: r = -0.368, P = 0.00670; UMBR: r = -0.480, P = 0.0002; BYCONE: r = -0.382, P = 0.0052).

DISCUSSION

Novel object tests are often used in horses to test their temperamental characteristics. In addition, these tests might be used as a part of a welfare assessment (Wageningen UR Livestock Research). The umbrella is a very popular item during novel object tests (Visser et al., 2008; Leiner and Fendt, 2011) but a traffic cone might also be used (Malmkvist et al., 2012). These items are regularly used as horses react strongly to them. The strength of the reaction might be important when a novel object tests is used to assess welfare in horses. In order to strengthen the horse's reaction, a moving object might be used (Malmkvist et al., 2012). The results of our study showed that horses displayed more object related behaviors during the test with the black umbrella. Color and shape differences might affect the horse's reaction towards the objects. Christensen et al. (2008) found that horses generalized between objects of varying shapes with the same color, but they did not generalize when these objects had different colors. Two objects with the same shape but different color were used in our study (traffic cone), but no difference in reaction was seen. Hall and Cassaday (2006) reported a color effect and noted a more pronounced reaction of horses towards yellow, blue, white and black mats, compared to green, brown, red and grey mats. Consequently, the black-yellow striped cone should have caused a more pronounced reaction than the red-white striped cone but this was not the case. The black umbrella caused the most pronounced reaction, which might be related to the color of this item, as previous research noted that horses reacted stronger towards black mats (Hall and Cassaday, 2006). Previous research on the effect of a cardboard stripe on the ground in an alleyway test, concluded that both color and size affected

the horse's reaction (Saslow, 1999). However, when looking at correlations between behavior in presence of the objects and undisturbed behavior at stable, similar results are obtained for all four objects. The most pronounced reaction which is seen in presence of the umbrella might be caused by the size of this object as it is just bigger so more difficult to ignore. Indeed, Anderson et al. (1999) noted that opening an umbrella in front of a horse caused the most pronounced reaction compared to a popping balloon and a moving toy during a reactivity test.

Younger horses showed more object related behavior during the novel object test than older horses. Younger animals might be more curious compared to older ones. The absence of a reaction could suggest non-curiosity or indifference. The influence of age is also observed in research concerning relationships between behavior and health of horses. According to Burn et al. (2010) and Popescu and Diugan (2013), older horses are more likely to show unresponsiveness during a human approach test. Habituation to different situations due to their maturity explains this finding (Popescu and Diugan, 2013). Malmkvist et al. (2012) responded to this by adding movement to the object, as the horses in their study were older compared to horses used in previous novel object tests.

When analyzing sniffing and nibbling the object, influences from housing factors are seen. Nibbling was observed more frequently in small stalls, while sniffing occurred more in larger stalls. Nibbling shows exploration towards the objects and thus show the horse's curiosity and the absence of fear (Valenchon et al., 2013). Horses are restricted in their movements in smaller stalls and consequently exhibit more rapid this type of explorative behavior. The social aspect of the stall influenced sniffing during the novel object test, as it was seen more in horses housed in stalls with both visual and physical contact. Sniffing behavior is considered to reflect a positive reaction towards the object and in this study it is associated with specific features of housing, namely a large stall and visual/physical contact.

To determine associations between the horse's reaction during the novel object test and its behavior at stable and thus to verify whether similar results are obtained from all four objects, correlations were studied. Horses touching all objects frequently showed a high level of bedding related behavior during undisturbed behavior observations. This association was found in the presence of all objects. The latter behavior is sometimes considered as a reflection of frustration. It seems to be influenced by the motivation to eat and increases after feeding when feeding time is delayed (Hughes and Duncan, 1998; Ninomiya et al., 2004). However in this study this result might be related to the experimental set-up. As baseline behavior profiling and novel object tests were performed at the same time of day for each horse, external environmental parameters were in both cases more or less the same. When hay is available, horses are not inclined to explore their environment, resulting in less contact with the object. Indeed, exploratory behavior decreases when horses are more satisfied in terms of eating motivation (Ninomiya et al., 2007; Freire et al., 2009). Visser et al. (2008) suggested that horses showing a high level of contact with the novel object, were very restless and active in their stable. Nevertheless, contact with the object (sniffing and nibbling) during a novel object test is considered as positive, as it indicates the absence of fear (Valenchon et al., 2013). However, when performing the novel object test in a stall, it seems that these environmental parameters should be taken into account.

Behavior during which a horse does not directly interact with the objects, may indicate the presence of fear or stress. For example snorting is often considered as a stress-related behavior (Visser et al., 2008). In addition, rearing and focusing might indicate the presence of fear. The presence of these behaviors is associated with vocalization during undisturbed behavior observations. A higher frequency of vocalization is observed when horses are isolated and confined for short or longer periods (Kiley-Worthington, 1990, Bagshaw et al., 1994), since this behavior occurs when social partners are separated (Waring, 1983). Thus this

behavior might indicate a higher level of stress. Furthermore, horses experiencing a higher level of fear or stress in their stall, show more fear responses during a novel object test. In addition, defecation might also reflect fearfulness during the novel object test, as the frequency of this behavior increases when horses are isolated and confined (Kiley-Worthington, 1990; Bagshaw et al., 1994). This behavior also correlated with vocalization during undisturbed behavior observations. As previously mentioned, the presence of roughage might influence the horse's reaction. If this is the case, it seems that the focus should be on the presence of fear reactions, such as rearing, not approaching the object or defecating, in order to correctly determine the animal's welfare state.

CONCLUSION

Horses showed a more pronounced reaction in the presence of the black umbrella during the novel object test, possibly caused by the size of this object. The results show that associations between behavior during the novel object test and behavior at stable were present. However, the presence of roughage influences the horse's reaction and should be taken into account. In this case, the focus should be on the presence of fear reactions, such as rearing, not approaching the object or defecating, in order to correctly determine the animal's welfare state.

ACKNOWLEDGEMENTS

We would like to thank the riding school for giving permission to carry out the observations at their school.

Conflict of interest: There is no conflict of interest in this manuscript.

The idea for the paper was conceived by Bert Driessen. The experiments were designed by Anneleen Bulens, Jos Van Thielen, and Bert Driessen and performed by Hanne Sterken. The

data were analyzed by Anneleen Bulens and Sanne Van Beirendonck. The paper was written by Anneleen Bulens, Sanne Van Beirendonck, Jos Van Thielen, and Bert Driessen. Funding was provided by KU Leuven and Thomas More. Animals were treated in accordance with the regulations of the Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes (OJEU, 1986).

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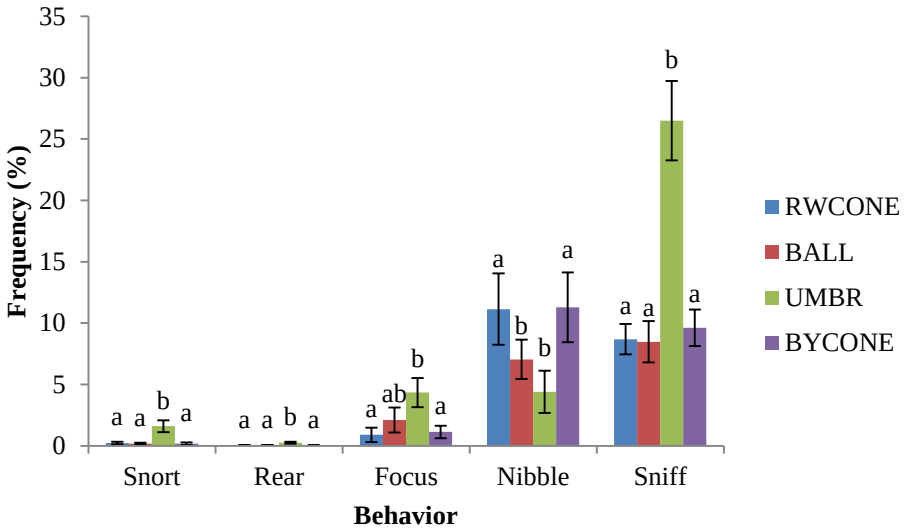

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312 **Figure 1** The frequency of object-related behavior (Mean ± S.E.M.) in presence of the four
313 different objects. Significant differences ($P < 0.05$) are indicated with different letters per
314 behavior.

315 **Table 1** Overview of the number of horses per riding school.

Riding school/Gender	Mare	Gelding
1	4	8
2	6	6
3	4	4
4	5	7
5	6	6

316 **Table 2** Overview of the environmental parameters and their levels.

Parameter	Levels
Stall size	1: Small < (2 x height of the horse)^2 m^2
	2: Medium (2 x height of the horse)^2 m^2
	3: Large > (2 x height of the horse)^2 m^2
Social contact	1: Visual social contact: bars only in front, with an opening for the horse's head
	2: Visual and physical contact: bars in front and in one or more sidewalls
Presence of feed	1: Yes
	2: No

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318 **Table 3** Definition of the behaviors used during control observations and novel object tests.
319 Behaviors indicated with « NOT » were only observed during novel object tests (Visser et al.,
320 2008; Christensen et al., 2011; Malmkvist et al., 2012;).

Behavior	Definition
Standing alert	Elevated neck and head, ears pricked. In NOT distinguishing between orientation of the head: "standing alert" is noted when the head is in other directions then the novel object.
Dozing	Standing inattentively with head and neck lowered, eyes closed and ears relaxed
Eat	Eating concentrates
Drink	Mouth in contact with the water dispenser for more than 5 s
Defecation	Defecating
Vocalization	Vocalizing

Locomotion	Horizontal movement of the body, four-time gait
Stereotypic behavior	A uniform pattern of movement apparently without purpose (weaving, head shaking, crib-biting)
Paw	One foreleg extended quickly forward, followed by movement backward, dragging the toe against the ground in a digging motion
Kick	The horse lifts its weight on its forelegs and extends one or both hind legs in a rapid motion
Snorting (NOT)	Forceful expulsion of air through the nostrils incidentally preceded by a raspy inhalation sound
Focus (NOT)	Focused on novel object (ears, eyes and head pointed in direction of novel object)
Nibbling (NOT)	Exploring the novel object by nibbling
Sniffing (NOT)	Standing with lowered head and nostrils within 10 cm of object; repeated and obvious exhalations
Rear (NOT)	The horse rears more than 50 cm and bears its weight on the hind legs

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323 **Table 4** Influence of box size and type of social contact on specific behavior during the novel
324 object tests. Significant differences ($P < 0.05$) are indicated with different letters per behavior.

Behavior	Parameter	Mean (%)	S.E.M.	P-value
Nibbling	Small box	17.38^{a}	2.97	0.0018
	Medium box	2.06^{b}	2.83	
	Large box	7.64^{b}	2.47	
Sniffing	Small box	7.80^{a}	2.79	0.0013

	Medium box	13.08[b]	2.85	
	Large box	19.85[b]	2.05	
Sniffing	Visual contact	9.21[a]	1.95	0.0225
	Visual and physical contact	17.94[b]	2.56	

325